



Alien populations of painted frogs, genus *Discoglossus*, on the southeastern coast of France: two examples of anthropogenic introduction

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Abstract.—Introductions of animals and plants by humans permanently restructure the distribution ranges of species and the compositions of communities, a phenomenon which has been intensified in recent decades with globalization. However, it is often difficult to date these introductions or to identify the geographic origin of the introduced individuals. In this study, genetic variation in the mitochondrial gene for cytochrome *b* was examined in native populations of painted frogs (genus *Discoglossus*) and introduced individuals discovered at two novel locations in the south-east of France, to determine their specific ranks and origins. The population of *Discoglossus sardus* identified at Marseille probably originated from Corsica, and that of *Discoglossus pictus* discovered at Grimaud in the Var Department probably originated from the previously introduced range of the species in the southwestern Mediterranean region of France. These newly discovered populations of painted frogs represent an unresolved conservation issue, as they are allochthonous in the respective regions on one hand, but on the other hand they belong to species which are legally protected in France and Europe. As next steps, assessing their range expansion is important, as is studying the nature of the relationship between these painted frog populations and the native amphibian communities.

Keywords. Anura, biogeography, conservation, human introduction, invasive capacity, native range

Résumé.—Les introductions humaines d'animaux et de plantes restructurent en permanence l'aire de répartition des espèces et la composition des communautés, phénomène qui s'est intensifié ces dernières décennies avec la mondialisation. Cependant, il est souvent difficile de dater ces introductions et d'identifier leurs origines. Dans cette étude, la variation génétique du gène mitochondrial du cytochrome *b* a été examinée dans des populations indigènes de discoglosses (genre *Discoglossus*) et chez des individus introduits, découverts sur deux localités inédites dans le sud-est de la France. L'objectif étant de déterminer le rang spécifique et l'origine des discoglosses observés sur ces nouvelles localités. Les analyses témoignent de la présence de *Discoglossus sardus* (probablement originaire de Corse) à Marseille et de *Discoglossus pictus* (provenant probablement de l'aire d'introduction de l'espèce située dans le sud-ouest de la région méditerranéenne française) à Grimaud dans le département du Var. Ces populations nouvellement découvertes représentent un problème de conservation non résolu, car elles sont d'une part allochtones dans les localités respectives, mais d'autre part appartiennent à des espèces légalement protégées en France et en Europe. À l'avenir, il sera important d'évaluer leur expansion géographique et d'étudier la nature de la relation entre ces populations de discoglosses et les communautés d'amphibiens indigènes.

Mots clés. Anoure, biogéographie, conservation, introduction humaine, capacité d'invasion, aire de répartition d'origine

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Introduction

The presence of barriers, both natural (e.g., rivers, sea, mountains) and artificial (e.g., roads, urban centers), often limits the dispersal of terrestrial vertebrates (Peres et al. 1996; Epps et al. 2005; Riley et al. 2006; Delaney et al. 2010; Chiari et al. 2012). However, by their historic and contemporary activities, such as transport, international trade, experiments, agriculture, etc., humans have caused the introduction of species into territories far from their original distribution (Pyšek et al. 2010). This phenomenon has been exacerbated in recent decades due to globalization, which has intensified terrestrial, aerial, and sea transport, and increased international trade (Levine and D'Antonio 2003; Westphal et al. 2008; Hulme 2009). Many organisms, including amphibians, are affected by these anthropic introductions (either accidental or voluntary), which are often characterized by high potential population growth rates, allowing the introduced species to become permanently established (Kraus 2015; Aellen et al. 2017).

In France, numerous non-native amphibian species have settled successfully. Among the more historical introductions (i.e., in the early 20th century) are *Discoglossus pictus* in Banyuls-sur-Mer (Pyrénées-Orientales) and probably *Triturus carnifex*, which was introduced in Chêne-Bourg (Switzerland) [Lescure and de Massary 2012] very near the border with France and is now present in Ain and Haute-Savoie, around Lemane Lake (Arntzen 2001; Dufresnes et al. 2016). During the mid-20th century, *Pelophylax bergeri* was translocated from Central Italy multiple times, resulting in introgressive hybridization with native populations of its sister taxon *Pelophylax lessonae* (Dufresnes et al. 2017). During this time, massive importations of several other *Pelophylax* species (e.g., *P. bedriagae*) for human consumption also occurred (Pagano et al. 2003).

More recently, *Lithobates catesbeianus* was introduced in Arveyres (Gironde), *Xenopus laevis* in Bouillé-Saint-Paul (Deux-Sèvres), *Bombina bombina* in Albestroff (Moselle), and *Eleutherodactylus johnstonei* in the urban zone of Nantes (Loire-Atlantique) [Lescure and de Massary 2012; Labadesse and Eggert 2018]. In

addition, some indigenous species have been translocated within France, such as *Hyla meridionalis* into Hyères Islands (Knoepffler 1961), *Ichthyosaura alpestris* on the limestone plateau of Larzac (Hérault) [Denoël 2005; Geniez and Cheylan 2012], and *Speleomantes strinatii* into a mine in the French Pyrénées (Ariège) [Lunghi et al. 2018] and a cave near Angles-sur-l'Anglin (Vienne) [Lucente et al. 2016].

This report documents two additional cases, based on observations of painted frogs (*Discoglossus*) between 2011 and 2018 at two continental localities in the south-east of France, in the city of Marseille (Bouches-du-Rhône Department), and in a plain and semi-urban zone in the locality of Grimaud (Var Department) [Table 1, Fig. 1]. These observations have generated strong interest because the localities are geographically distant from the documented ranges of the two species of *Discoglossus* known to be present in France (Fig. 1). *Discoglossus sardus* is distributed in Sardinia, in the Tuscan Archipelago and the adjacent Italian coast, and in France in the eastern part of Hyères Islands (Port-Cros and the Levant Islands) and Corsica (Delaugerre and Cheylan 1992; Lescure and de Massary 2012). The other species, *Discoglossus pictus*, is indigenous to North Africa (Algeria and Tunisia), Sicily, Malta, and Gozo (Sindaco et al. 2006). However, since *D. pictus* was originally introduced into France in the department of Pyrenees-Orientales, it has colonized the adjacent departments of Aude, Hérault (Knoepffler 1962; Fradet and Geniez 2004; Geniez and Cheylan 2012), and the extreme north-east of Spain (Franch et al. 2007).

Because of the difficulty in unambiguously identifying species of *Discoglossus* by morphological criteria alone, molecular phylogenetic analyses were conducted to assess the species identity and geographic origins of the observed painted frogs from the two novel locations in mainland France.

Materials and Methods

Genetic samples. Tissue samples and buccal swabs were taken on 31 May 2018 and 17 June 2018, respectively, from nine tadpoles from Marseille; and on 7 November

Table 1. Available data on the occurrences of the two introduced species of *Discoglossus* on the southeastern coast of France. Ind. = Indeterminate.

Species	Date	Locality	Latitude (N)	Longitude (E)	Number of specimens	Observers
<i>Discoglossus sardus</i>	17 June 2011	Marseille	43°20'47.3"	5°26'10.9"	3–4	A. Piquet
	17 June 2015	Marseille	43°20'46.5"	5°26'22.4"	1	V. Mariani
	31 March 2018	Marseille	43°20'20.9"	5°26'32.4"	3	M. Policain
	16 April 2018	Marseille	43°21'00.7"	5°26'12.5"	60	M. Policain and F. Grimal
<i>Discoglossus pictus</i>	2016–2017	Grimaud	43°16'32.2"	6°31'52.9"	Ind.	V. Fradet and A. Dubois
	2016–2017	Grimaud	43°16'59.0"	6°30'59.8"	Ind.	V. Fradet and A. Dubois
	2016–2017	Grimaud	43°16'12.7"	6°33'02.6"	Ind.	V. Fradet and A. Dubois
	7 November 2018	Grimaud	43°15'52.6"	6°33'39.1"	3	J. Renet, M. Policain, and M. Marmier

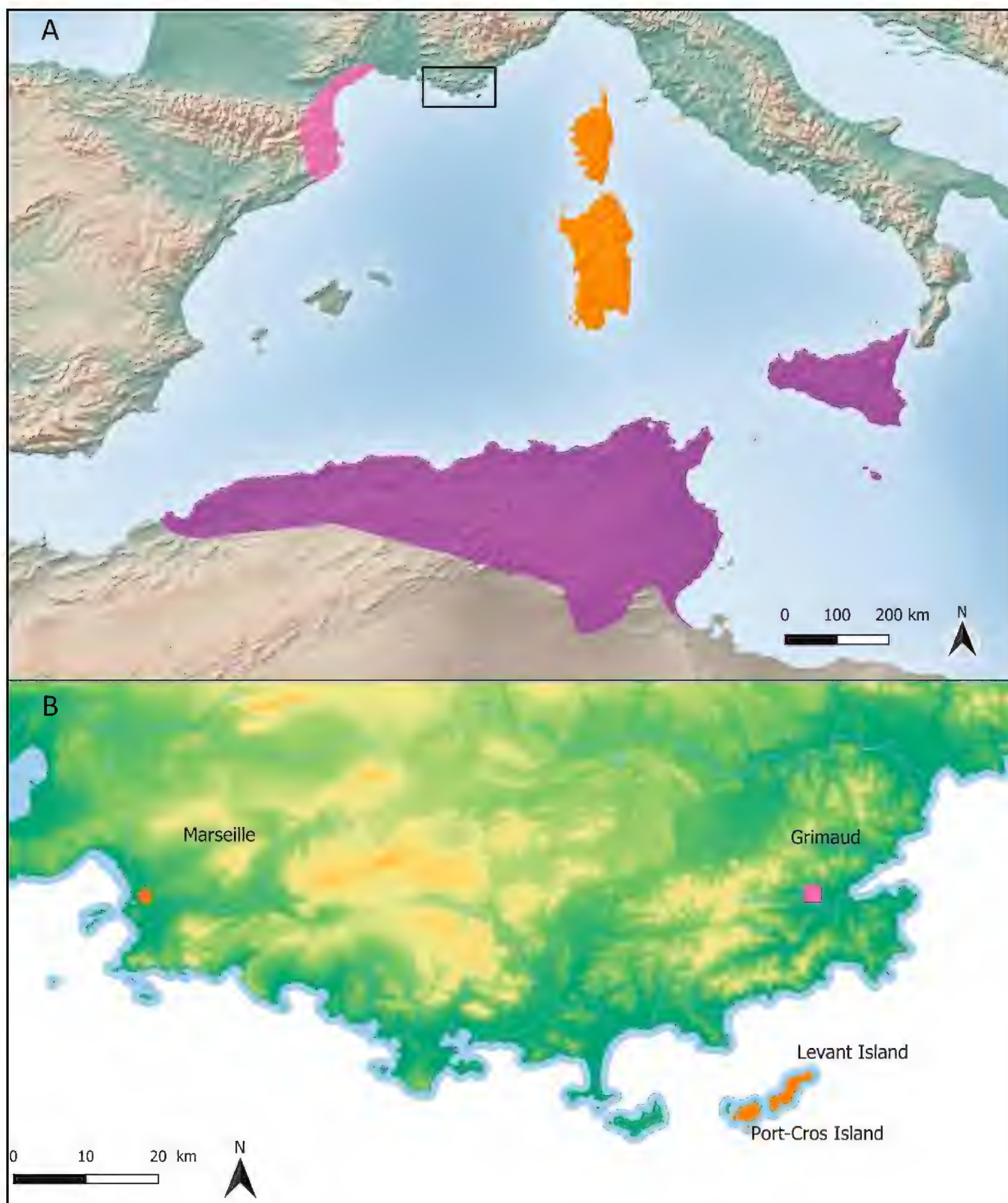


Fig. 1. (A) Map of the ranges of *D. sardus* (orange: native population range) and *D. pictus* (purple: original distribution range; pink: introduced population range). (B) Enlarged view of the area with the newly introduced populations of the two species in southern France (orange star: *D. sardus* in Marseille; pink square: *D. pictus* in Grimaud), which is indicated by the black rectangle in (A).

2018 from two adult specimens from Grimaud (Fig. 2C). The sampled individuals were collected at night along two small shady streams and in a water-filled moat bordering a wasteland and a vineyard (Fig. 2B,D). Additional comparative samples were collected from various sites in Sardinia, Corsica, and the Hyères Archipelago (Port-Cros), in the form of either muscle tissue samples from roadkill specimens or tail tips of tadpoles.

Genetic analyses. Total genomic DNA was extracted from the buccal swabs and tissue samples using a salt extraction protocol (Bruford et al. 1992). A fragment of the mitochondrial gene for cytochrome *b* (*cob*) was amplified using the primers in Zangari et al. (2006): MVZ15-L

(GAACTAATGGCCCACACWWTACGNAA) and H15149-H (AAACTGCAGCCCCTCAGAATGATATTGTCCTCA). As these primers did not reliably amplify the respective fragment, particularly in *D. sardus*, most samples were also amplified using two newly developed specific primers: Dsard-Fwd (TGACCTACCTACCCCATCCA) and Dsard-Rev (GGGCAGTACGTAGCCTACAA). For both primer pairs, the PCR protocol consisted of an initial step of 90 sec at 94 °C, followed by 35 steps of 94 °C (30 sec), 53 °C (45 sec), 72 °C (90 sec), and a final elongation step of 10 min at 72 °C. PCR products were treated with exonuclease I (New England Biolabs) and shrimp alkaline phosphatase (Promega) to inactivate remaining



Fig. 2. (A) Adult male *Discoglossus sardus* from Marseille, 31 May 2018. (B) *Discoglossus sardus* habitat in the city of Marseille. (C) Adult *Discoglossus pictus* from Grimaud, 7 November 2018. (D) *Discoglossus pictus* habitat in Grimaud. The white arrow indicates the position of a ditch filled with water, where three individuals were observed. Photos by Mathieu Policain (A–B), Julien Renet (C), and Google Map/Street View (D).

primers and dNTPs, and then sent for sequencing to LGC Genomics (Berlin, Germany). Chromatographs were checked and obvious errors in automated sequence reads were corrected using Codon-Code Aligner (v2.0.6, Codon Code Corporation). All newly determined sequences were submitted to GenBank (accession numbers MT569346–MT569387).

The cytochrome *b* fragment used was chosen to allow comparisons with the results of Zangari et al. (2006), who published sequences of *D. sardus* and all other species in the genus from various localities. These sequences were downloaded from GenBank and trimmed to match the shorter length of the sequences produced using the specific *D. sardus* primer pairs listed above. Note that this fragment is not homologous with the one used by Martínez-Solano (2004) and Vences et al. (2014), and therefore direct comparisons with the results of those studies (which focused on *D. galganoi* and *D. pictus*) are not possible.

Sequences were aligned and phylogenetic analysis was conducted using MEGA, v. 7 (Kumar et al. 2016). The sequences were first aligned using the Clustal algorithm, then the appropriate substitution model (Kimura-2-parameter + G) was selected under the Akaike Information Criterion, phylogenetic trees were subsequently inferred under the Maximum Likelihood

(ML) optimality criterion with NNI branch swapping, and node support was assessed with 500 bootstrap replicates. The tree was rooted with *D. montalentii*, which represents the sister species to all other *Discoglossus* (Zangari et al. 2006; Pabijan et al. 2012; Biton et al. 2013; Dufresnes et al. 2020).

Results and Discussion

Genetic Identity of the New *Discoglossus* Populations

The Maximum Likelihood tree reconstructed from the 258 bp cytochrome *b* segment retained for analysis (Fig. 3) recovered phylogenetic relationships among *Discoglossus* species which were largely similar to those of more comprehensive, multi-gene studies (Zangari et al. 2006; Pabijan et al. 2012; Biton et al. 2013; Dufresnes et al. 2020). However, as expected from such a short gene fragment, the relationships among most species were not reliably resolved. All species of *Discoglossus* were recovered as monophyletic groups, with bootstrap supports of 90–99%.

For the new continental French populations that are the focus of the present study, the tree is unambiguous in placing the samples from Marseille into the *D. sardus* clade, and the samples from Grimaud into the *D. pictus*

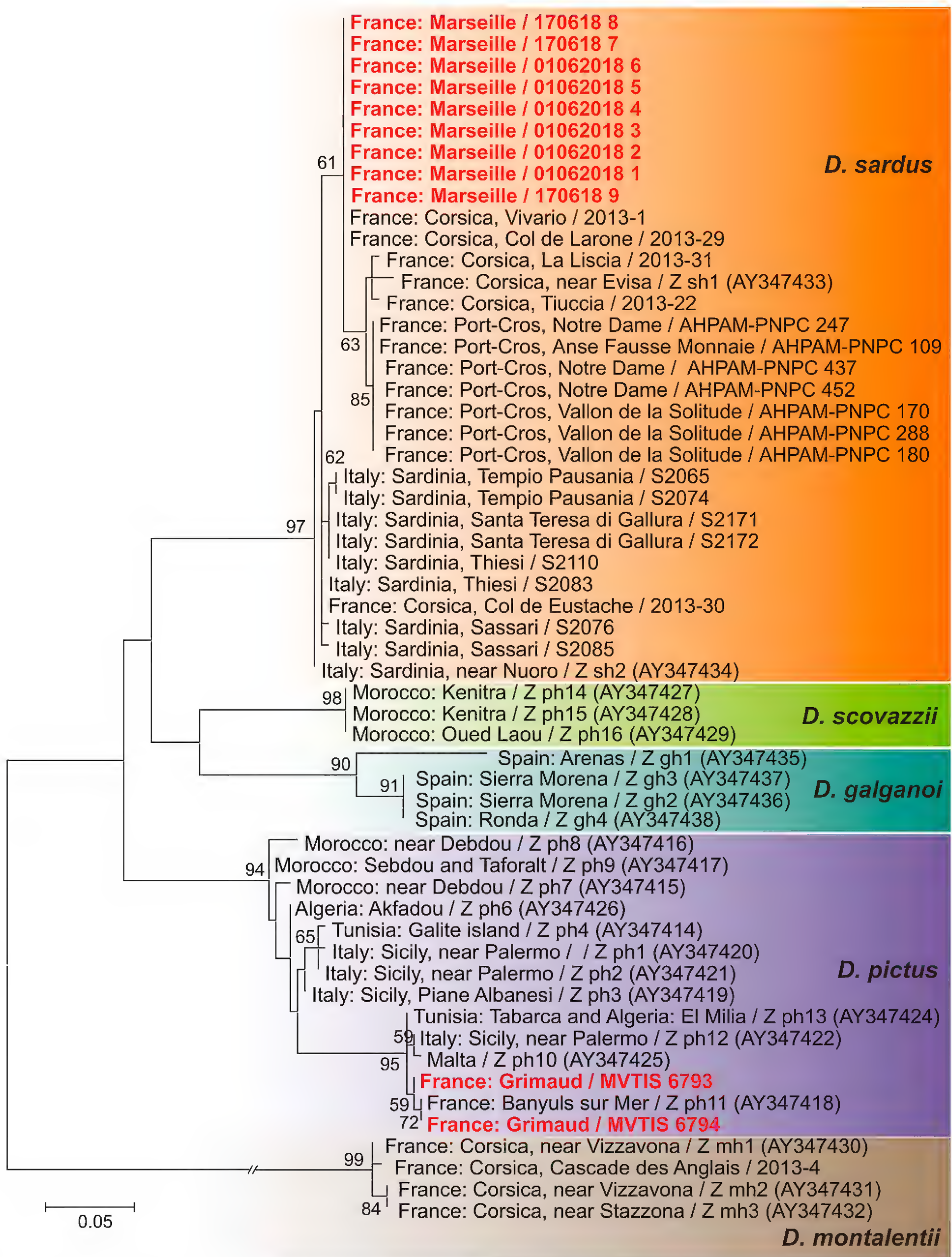


Fig. 3. Maximum Likelihood tree of *Discoglossus* based on a 258 bp fragment of the mitochondrial cytochrome *b* gene. Numbers at nodes are bootstrap values (500 pseudoreplicates) in percent. After the locality, sample numbers are given, including GenBank accession numbers in parentheses for those sequences taken from GenBank. “Z” marks sequences from the work of Zangari et al. (2006). Samples from the two newly discovered introduced populations are highlighted in bold, red font.

clade. All nine *D. sardus* specimens sequenced from Marseille had identical sequences, and the same haplotype was also found in two localities in Corsica. In contrast, all sequenced specimens from the Hyères Archipelago and from Sardinia differed by at least three mutations, suggesting that the Marseille population most likely originated by the introduction of only a few individuals from Corsica. Of the two specimens from Grimaud, one had a haplotype identical to that of a specimen from Banyuls-sur-Mer, while the second one differed by a single mutation. This suggests a probable origin of this population by introduction from the invasive range of *D. pictus* in the southwestern French Mediterranean region.

The tree generated here also recapitulates the surprising finding of Zangari et al. (2006) regarding the presence of rather distinct mitochondrial haplotypes of *D. pictus* in Sicily. It also reveals that *D. sardus* from Corsica and Sardinia are not reciprocally monophyletic based on mitochondrial DNA. In this latter case, the one Corsican *D. sardus* (from Col d'Eustache) clustering among the Sardinian haplotypes was sequenced several months before the samples from Sardinia were processed, which excludes the possibility of an artifact due to a mislabelled sample or contamination.

Geographic Origin and Status of the New *Discoglossus* Populations

The results of this analysis shed a new light on the ranges of the two species of *Discoglossus* that are present in the south of France. In the population of *D. sardus* established in Marseille represents the second known mainland population, after the population of Monte Argentario peninsula (Tuscany, Italy). The genetic similarity of the Marseille samples with those from the two Corsican localities allow us to refute the hypothesis of an ancient relict population naturally occurring in Marseille. In such a case we would expect genetic relationships with the individuals from Port-Cros or the Levant islands (Hyères Archipelago), which are geographically much closer to Marseille than Corsica. On the contrary, an introduction from Corsica is consistent with the intensity of the maritime traffic between Corsica and Marseille, and the apparent lack of genetic diversity in the Marseille samples is also in agreement with an introduced origin. Even if the date of introduction of this species cannot be determined, its spread over an area of approximately 0.66 km² suggests that the arrival of *D. sardus* in Marseille is not very recent.

With a geographic extension of almost 210 km to the east (i.e., the distance between the easternmost population known to date and the recently discovered population at Grimaud), and its crossing of the Rhone River, the anthropic introduction of *D. pictus* is beyond doubt. Its arrival in Grimaud, along the La Garde river, could be linked with the trade activities of the many nurseries and garden stores (12 shops identified within

10 km of the sampling locality), which are known to be vectors of various species introductions worldwide (e.g., anurans, snails, plants; Christy et al. 2007; Bergey et al. 2014).

The two newly detected introductions of *Discoglossus* in continental France could have been accidental, or they could have been deliberate due to a variety of motivations, such as experimental studies on naturalization conducted in the past, or the liberation of captive animals. For instance, *D. sardus* tadpoles from Port-Cros Island were introduced into a tributary of la Mole river (Var) as an experiment in 1955, and this attempt at establishing a reproducing population is known to have succeeded at least until 1959 (Knoepffler 1962).

The discoveries of these new populations testify once again that today the natural elements, such as rivers or oceans, do not represent absolute barriers for either native or allochthonous species. Invasion success generally depends more on the ability of a species to respond to natural selection than on broad physiological tolerance or plasticity (Lee 2002). In the present case, considering the ranges of these two species and their reproductive status, it seems that they can be considered as successful colonizers. In fact, more comprehensive phylogeographic studies of *D. pictus* and *D. sardus* in the future should also examine the possibilities of *D. sardus* translocations among Corsica and Sardinia (given the clustering of the one Corsican haplotype among the Sardinian haplotypes; Fig. 3) and of *D. pictus* to or from Sicily (given the presence of highly distinct haplotypes on this island; Fig. 3).

Conservation Issues

Williamson (1996) considers that a biological invasion occurs when an organism takes root outside of its indigenous range. The IUCN Invasive Species Specialist Group proposes a more specific definition—that a biological invasion has occurred as soon as an introduced species is a factor of damage and affects the local biodiversity. In fact, it is important to distinguish between an allochthonous species introduced by humans, which is inoffensive in many cases, and an invasive species, which, by definition, is not only introduced outside of its indigenous range but also exerts a negative impact on biodiversity and more globally on the ecosystem (Lambertini et al. 2011).

In the urban and sub-optimal ecological context of the city of Marseille, the population of *D. sardus* probably does not represent a threat to the ecosystem, which is *a priori* of 'low ecological value.' Furthermore, this population is already threatened by a large-scale urban development project. Although *D. sardus* is considered to be Least Concern in both the IUCN Red List and the National French Red List (Andreone et al. 2009; UICN France et al. 2015), the global assessment has determined a decreasing population trend. This points to an important

and challenging dilemma highlighted by Marchetti and Engstrom (2016): how to manage allochthonous, or even invasive species, that are threatened (or may become threatened in the future) in their native range? Several authors (e.g., Marris 2014; Heise 2018) have suggested pragmatic approaches when dealing with non-native species, especially in urban environments which indeed could become sanctuaries for many species (native or not) that are threatened in their original habitat. Especially with shifting ranges due to climatic change, the distinctions between native and non-native will become increasingly vague, and human-aided translocations of some threatened species are already being discussed (Egan et al. 2018).

These elements lead us to consider the presence of these new *Discoglossus* populations as a high-priority conservation issue. We can also add that *D. sardus* is assessed as Threatened in the Var Department (cat. VU IUCN Redlist) [Marchand et al. 2017], and as threatened with extinction at Port-Cros Island, Port-Cros National Park (Duguet et al. 2019).

Concerning *D. pictus*, the question of its biological status requires more scrutiny because other authors have attributed an invasive nature with a high rate of dispersal to this species (Montori et al. 2007). Its invasive capacity does not seem to be related to its adaptive advantages, but rather to the suitability of local abiotic conditions (Escoriza et al. 2014). The modeling of its potential habitat conducted by Escoriza et al. (2014) includes areas that are geographically near the locality of Grimaud, and incorporation of the new occurrences should allow an adjustment of the predictive models. Furthermore, the potential area of this species should be considered as wider than suggested by previous models. In any case, the expansion of *D. pictus* from a single location in Banyuls-sur-Mer, Eastern Pyrénées a century ago (see Wintrebert 1908) is not an artifact; i.e., it represents a natural range expansion (Pujol-Buxó et al. 2019a) into a currently occupied area in France and Catalonia of more than 10,000 km² (Montori et al. 2009). A negative impact of this species on co-occurring anurans (e.g., *Pelodytes punctatus* and *Epidalea calamita*) has been suspected (Escoriza and Boix 2012, 2014; Richter-Boix et al. 2013; San Sebastián et al. 2015). However, this possibility requires further study as some have hypothesized that temporal or evolutionary changes may have moderated the effects and disturbance of *D. pictus* on native species (Pujol-Buxó et al. 2019b).

In any event, according to the actual current French regulations, all individuals of both species, as well as their “core” habitat, are strictly protected by a ministerial order (DEVN0766175A). Although a recent update of this order would specifically exclude *D. pictus*, we hope for the continued regulatory protection of *D. pictus* in French territory. Given the similarities in biotic features between the source and recipient communities (Escoriza and Ruhí 2016), we suspect that *Discoglossus* species

are probably not harmful to the local French anuran communities, and we therefore do not recommend the eradication of their non-native populations.

Lastly, to better manage this situation going forward, we recommend a monitoring program to: (1) characterize a predictable range expansion of these two painted frog species in adjacent localities; and (2) implement complementary studies in order to better assess the nature of the relationship between these introduced species and the native amphibian communities.

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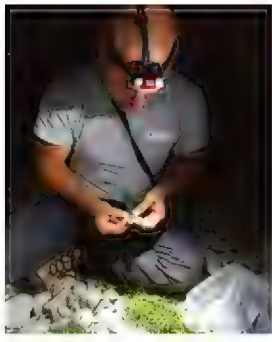
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Anthropogenic introduction of *Discoglossus* in southern France



Julien Renet is a French wildlife biologist at the NGO Conservatoire d'espaces naturels de Provence Alpes Côte d'Azur (CEN PACA, <http://www.cen-paca.org/>), where he designs, coordinates, and implements the conservation programs for several species of herpetofauna (e.g., *Pelobates cultripes*, *Triturus cristatus*, *Emys orbicularis*, *Euleptes europaea*) of the Provence-Alps-Côte d'Azur region in France. His work concerns the general framework of conservation biology, more specifically ecology, biogeography, population dynamics, and assessments of non-invasive individual marking methods. Additional information about his work is available on his ResearchGate page, at: https://www.researchgate.net/profile/Julien_Renet/research.



Rémi Duguet is a private consultant for biodiversity assessments and monitoring, and a part-time teacher of herpetology at the University of Franche-Comté, Besançon, France. Rémi has worked for many years in amphibian and reptile conservation, especially in France and in the western Indian Ocean islands. He edited the book *Les Amphibiens de France, Belgique et Luxembourg*, published in 2003 by Biotope (Meze, France).



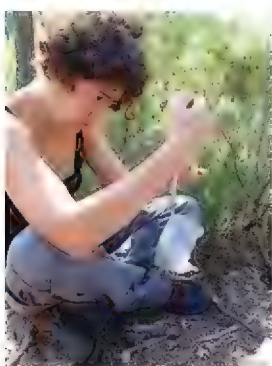
Mathieu Policain is a naturalist who has worked for the NGO Colinéo-Assenemce (Marseille, France, <https://colineo.fr/>) for five years. He has a special interest in the Mediterranean herpetofauna, and is currently an active volunteer in various efforts to protect amphibians and reptiles.



Alison Piquet is a French herpetologist with a Master's degree and almost ten years of national and international experience in the field. Specializing in reptiles, and snakes in particular, she travels extensively to conduct inventory field studies and observe reptiles, amphibians, birds, and spiders in the wild. As a herpetologist, she joined the *Radeau des Cimes* international expedition to Laos in 2014, in order to inventory the herpetofauna in a poorly-known primary forest area. She also spent a few years in Australia studying and photographing the amazing wildlife of that country.



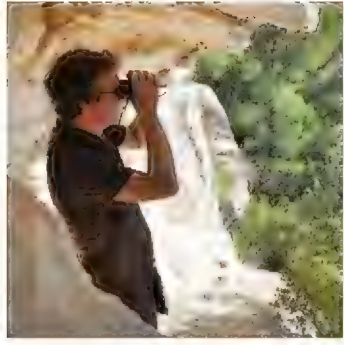
Vincent Fradet is an amphibian specialist who graduated from the École Pratique des Hautes Études (Paris-Sorbonne) where he studied *Discoglossus pictus* phylogeography. Vincent now works in the service of nature for several environmental NGOs.



Pauline Priol works as scientific consultant in conservation biology, has spent several years managing conservation programs for endangered species (*Emys orbicularis*, *Pelobates cultripes*), and obtained two graduate degrees from universities in France and Canada. Pauline is now working with field practitioners, various stakeholders, and statisticians to develop methods for modeling population dynamics, building and evaluating monitoring protocols, estimating demographic parameters, evaluating impacts of perturbations, and evaluating/defining management actions. Her specialty is herpetofauna (e.g., European pond turtles *Emys* and *Mauremys*, crested newts, *Discoglossus*, spadefoot toads, Mediterranean lizards) but she also works on birds (stock programs, woodcock), crayfish, and insects (dragonflies, butterflies).



Grégory Deso is a herpetologist who has been active in environmental organizations since 1999 on the Mascarene Islands, where his work has focused on the distribution and ecology of various native and introduced species and their interactions linked to human activity. He now resides in mainland France (Provence Alps Côte d'Azur region) where he founded the NGO Association Herpétologique de Provence Alpes Méditerranée (<https://ahpam.fr/>), which works toward the protection of amphibians and reptiles. Today, Gregory's work concerns all aspects of the continental and island Mediterranean herpetofauna.



François Grimal is a French wildlife biologist at the NGO Ligue pour la Protection des Oiseaux (LPO, <https://www.lpo.fr/>), an affiliate of Birdlife International. François designs and coordinates conservation and monitoring programs for several amphibian populations of the Provence-Alps-Côte d'Azur region, in particular *Epidalea calamita* and *Pelophylax* sp. His work concerns the ecology, population dynamics, implementation of genetic and bioacoustic studies, and photographic and individual marking methods.



Giuseppe Sotgiu is an Italian biologist who works as an independent researcher specializing in the conservation of the insular herpetofauna and ichthyofauna of Sardinia. The species he primarily studies is the Sardinian Newt, *Euproctus platycephalus*, one of the most endangered urodeles in Europe. Giuseppe collaborates with the Department of Zoology of the University of Sassari, Sardinia, Italy. Since 2007, he has also collaborated with the Institute of Zoology at the Zoological Society of London, in order to understand the impacts of pathogens such as chytridiomycete fungi on the amphibian populations in the Mediterranean islands. He is also interested in studying the impacts of alien species on native endemic species, and developing methods to mitigate the effects of ecological invasions.



Miguel Vences is a zoologist and evolutionary biologist at Braunschweig University of Technology, Germany. He leads a long-standing research program on amphibian and reptile biology, with investigations spanning classical taxonomy, molecular evolution, diversification processes, biogeography, and conservation biology. Miguel has worked extensively on the herpetofauna of Madagascar, and also on numerous taxa in Europe.